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Microfluidic Sample Preparation for Immunoassays

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ABSTRACT

Researchers at Lawrence Livermore National Laboratory are developing means to collect and identify fluid-based biological pathogens in the forms of proteins, viruses, and bacteria. To support detection instruments, we are developing a flexible fluidic sample preparation unit. The overall goal of this Microfluidic Module is to input a fluid sample, containing background particulates and potentially target compounds, and deliver a processed sample for detection. We are developing techniques for sample purification, mixing, and filtration that would be useful to many applications including immunologic and nucleic acid assays. Many of these fluidic functions are accomplished with acoustic radiation pressure or dielectrophoresis. We are integrating these technologies into packaged systems with pumps and valves to control fluid flow through the fluidic circuit.

Keywords: microfluidics, MEMS, sample handling, dielectrophoresis

1. INTRODUCTION

There are many applications of biological molecule detection that could benefit from improved sample handling. Medical clinicians are ever searching for faster, cheaper, and more sensitive, clinical diagnostic assays and instrumentation. Biomedical researchers are interested in drug discovery, nucleic acid sequencing, proteomics, etc. Similarly, in the interest of national security, government and military agencies are interested in detection of a wide variety of biological warfare agents. Researchers at Lawrence Livermore National Laboratory are developing means to collect and identify biological pathogens in the forms of proteins, viruses, and bacterias. To support detection devices, we are developing a flexible fluidic sample preparation unit that will autonomously process samples for downstream detection. We are exploring techniques for sample purification, mixing, and filtration that would be useful for immunologic and nucleic acid assays.

Several discrete components are being linked together and packaged into a compact fluidic system. Commercially available piezoelectric pumps pull samples and reagents from reservoirs and drive the fluids to detection. Samples can be filtered or concentrated through the use of acoustic filtration. This concentration technique creates pressure nodes that concentrate and trap particles in a fluid stream. Acoustic energy can also be used to efficiently mix reagents and samples with the advantage of improving assay kinetics. Another technique being explored for sample purification or concentration is Dielectrophoresis (DEP). DEP creates electric field gradients that can selectively trap specific particles through manipulation of the electrical stimulus. We have demonstrated sample purification by selective DEP capture of contaminants in a flowing fluid. These components are being packaged in a polydimethylsiloxane (PDMS) platform for ease of fabrication and utility. This MicroFluidic Module will perform sample preparation functions required by future detection platforms. Microfluidic sample processing will lead to miniaturized portable devices that decrease reagent requirements and improve assay sensitivity.

2. MATERIALS AND METHODS

We have designed an initial architecture for our MicroFluidic Module that assumes fluid-based sample introduction. Sample introduction may range from simple manual pipetting or spotting to more sophisticated automated techniques. We further assume that the introduced samples contain background material that at least complicates performance of the assay and at worst prevents performance of assays. Background materials may raise the level of background signal, interfere with binding of reagents and labels, or inhibit assay performance. Therefore, it is advantageous in many

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scenarios to purify samples prior to performance of assays and delivery to detection instruments. Also, concentration of target molecules may ease sample handling, conserve reagents, and raise detection sensitivity. To accomplish these goals, we are developing two primary technologies: acoustic particle-manipulation and dielectrophoresis.

2.1 Acoustic Devices

Acoustic radiation was generated from piezoelectric transducers and coupled into flow chambers made of glass or acrylic. The acoustic waves were used to manipulate particles suspended in a fluid. Acoustic radiation pressure exerts a force on suspended particles. Radiation pressure generated in a frequency matched chamber can produce standing waves. The pressure field forces particles to collect in the nodes or anti-nodes of the standing wave, which is useful for particle concentration and sample filtration. Uncoordinated (not tuned to cavity) generation of acoustic radiation can cause random particles motion, useful for fluid mixing. The acoustic force exerted on a particle can be expressed as,

$$F_{ac} = -4\pi/3\ R^3 kEA \sin(2kx) \qquad (1)$$

where R denotes the particle radius, k is the wave number, and E is the acoustic energy density [1-5]. The compressidensity factor, A, reflects the relative density and compressibility of the particle with respect to the ambient medium. The sign of A determines whether the particles move to nodes of high or low pressure. The acoustic system provides the ability to perform basic fluidic functions necessary in an analysis system, specifically filtration, fractionation, concentration, and mixing. Expensive components such as the electronics and piezoelectric transducers can be reused while inexpensive flow chambers can be discarded after each use or where contamination is a concern.

Acoustic Mixing

Mixing is a challenge in microfluidics since small channel dimensions make it difficult to create turbulence. Acoustic mixing brings with it the advantages of rapid mixing, no moving parts, and no need for external injection of fluids or nozzles to create turbulence. We have previously demonstrated increased mixing using acoustic radiation in fluid chambers[6]. Rectangular lead zirconate titanate (PZT) transducers were coupled to glass or plastic flow chambers. Piezoelectric transducers were driven at 15-40 V_{pp} and frequencies of 0.1-5 MHz. With increasing voltage applied to the transducers we demonstrated increased mixing and increased binding of Bacillus globigii (B.g.) spores to antibody coated 5 μm spheres.

Acoustic Concentration

Acoustic radiation was also used to concentrate particles into areas of pressure nodes. Figure 1 shows a drawing of the device. The chamber design allowed for fluid flow into the chamber from a syringe and out of the chamber into a waste reservoir. Flow rates up to 100 μl/min were produced by a syringe pump. Transducers were powered at 40 V_{pp} and 3.3 MHz. Polystyrene particles of 5 μm diameter were suspended in a solution of water containing red dye. A volume of suspended beads was delivered into the chamber. The acoustic transducers were excited, capturing and concentrating particles in the pressure nodes. The particles were then held in the pressure nodes while a volume of distilled water (no dye) was flushed into the chamber at flow rates up to 100 μl/min.

2.2 Dielectrophoresis

An alternative method of concentrating particles and retaining them in a flowing stream is the use of dielectrophoresis. The dielectrophoretic force results from the ability of a particle to become polarized in the presence of a non-uniform electric field. Particles in the field will be attracted to areas of high or low field gradient depending on its electrical properties relative to those of the suspending medium. This force is highly dependent on the electrical properties of both the suspending medium and the particles and can be developed in both AC and DC fields. These properties are a strong function of the frequency of excitation.

The dielectrophoretic force on a spherical particle can be expressed as:

$$F_{DEP} = 2\pi\varepsilon_m r^3 \mathrm{Re}\{f_m\} \nabla E_{rms}^2 \qquad (2)$$

where ε_m is the permittivity of the medium in which the particle is suspended, f_m is the Clausius-Mossoti factor, r is the particle radius and E is the electric field[7]. The Clausius-Mossoti factor is expressed as:

$$f_m = (\varepsilon_p^* - \varepsilon_m^*)/(\varepsilon_p^* + 2\varepsilon_m^*) \qquad (3)$$

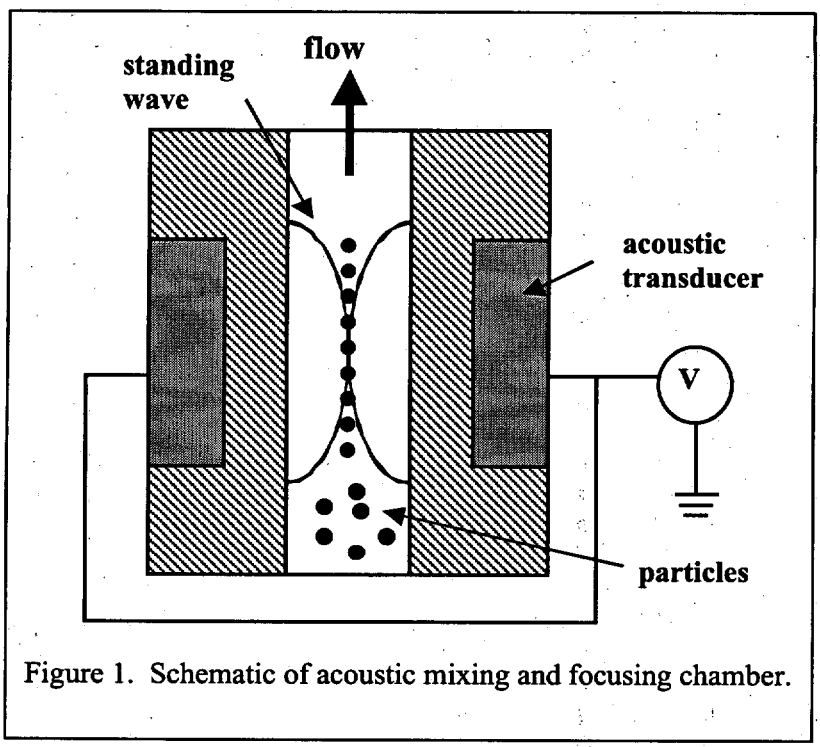


Figure 1. Schematic of acoustic mixing and focusing chamber.

where ε_p^* is the complex permittivity of the particle given by $\varepsilon^* = \varepsilon - j\sigma/\omega$ where ε is the permittivity, σ is the conductivity and ω is the angular frequency. Likewise, ε_m^* is the complex permittivity of the suspending medium. The Clausius-Mossoti factor for biological particles is a strong function of frequency. Typically, walled biological particles exhibit positive DEP at low frequencies in low conductivity solutions and negative DEP at higher frequencies. Cells without walls exhibit a mid-frequency region of positive DEP, while negative DEP is characteristic of low and high frequency operation[8]. Positive DEP is useful for the concentration of particles. The use of DEP forces becomes practical in microfluidic devices because sufficient field strengths are achievable at sub-millimeter dimensions with only a few volts applied to the electrodes.

A DEP chamber was fabricated to test the ability to pull particles from a flowing suspension. Devices consisted of interdigitated 30 μm-wide platinum electrodes fabricated on glass using standard photolithographic methods. A sample device is shown in Figure 2. The interdigitated electrodes were 2.2 cm long, 30 μm wide and spaced 30 μm apart. An AC voltage of 1-5 V_{rms} and 100 Hz-1 MHz was applied between the electrodes. Particles were observed under a 100x microscope for DEP trapping. Pumping was provided by piezoelectric micropumps (IMM, Mainz Germany) operating at 100 μl/min. Samples were pipetted into the inlet reservoir, pumped through the DEP chamber, and collected in the outlet reservoir. Samples were aspirated directly from the reservoir into a Microcyte (Optoflow, Oslo Norway) flow cytometer for quantification. The flow cytometer measures particles between 0.4 and 15 μm and concentrations of 10^2 to 10^7 particles/ml. Ten volumes of 1 μl were counted and averaged for each sample.

2.3 Pumps and Valves

We have begun implementing individual devices into complete packages. At the heart of any fluidic system is a method for pumping and steering fluid from input through detection. We have been evaluating a commercial micropump from IMM (Mainz, Germany) consisting of a piezoelectric actuator that operates on a membrane. The pumps are 12x12x3 mm and are capable of flow rates up to 400 μl/min and back pressures of 2 bar. We have developed simple leak-free compression fittings for mating the pumps into the fluidic package. This junction is advantageous because it is easy to disconnect and reconnect pumps when the fluidic circuit is to be discarded. The pumps are unidirectional meaning that either sample fluids flow through the pumps, or valves must be added and the circuit designed for bi-

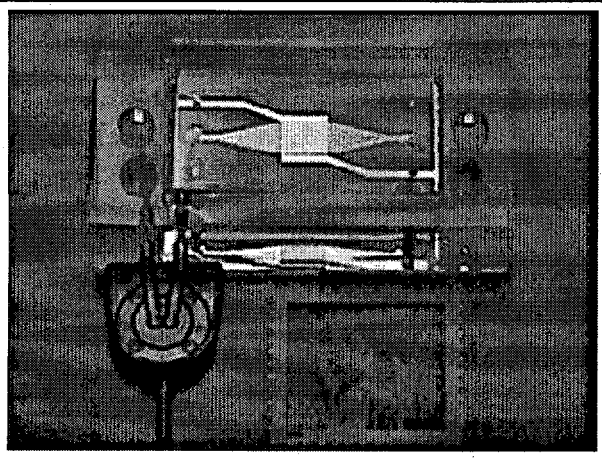


Figure 2. Dielectrophoresis chamber embedded in PDMS platform. Micropump, reservoirs and microchannels integrated into package.

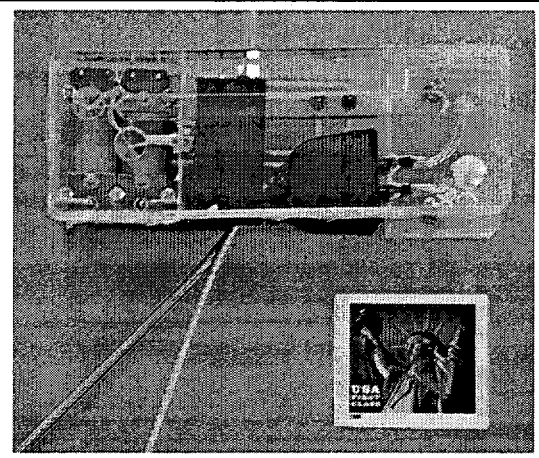


Figure 3. Top view of microvalve in PDMS. Pumps are visible to the right (viewed through the PDMS) and valve drivers to the left.

directional flow. For biological assays, it is not advisable to flow samples and reagents through the pump for fear of clogging and sample carryover. Therefore, we are developing microvalves which in combination with the pumps, can be operated similar to syringe pumps (Fig. 3). A fluid is drawn by the pump into a common reservoir. The inlet valve can be closed and an outlet valve opened. A second pump empties the reservoir. In this operation, the reservoir acts as the syringe, and no fluid flows through the pumps. Additional valves can be implemented for directing fluid through any number of downstream channels. We are currently characterizing the valves and the "syringe pump" to determine: flow rates, flow vs. back pressure, leakage pressure, switching rates, etc.

2.4 System Integration

We have developed microfluidic devices fabricated from a range of materials, most often enveloped in glass or acrylic. We have been using a PDMS (polydimethylsiloxane) elastomer for potting components and creating fluidic channels. This material allows us to embed many different materials including glass, metals, and polymers. PDMS is easier and cheaper to work with than glass and parts are produced faster. Connections to PDMS are easier to fabricate; connectors can be "polymerized" into the PDMS or compression connections can be molded into the PDMS. We have developed methods to fabricate microchannels, tees, and ells in the PDMS that have smooth profiles to maintain laminar flow and prevent dead volumes.

3. RESULTS

3.1 Acoustic Devices

Acoustic Mixing

It was observed from early mixing experiments with single transducers that areas of high particle velocity occurred at the corners of the piezoelectric transducers - the transition area between a region of high and low pressure. We consequently designed our chamber with multiple staggered transducers along the length of a flow channel, increasing the transition zones and permitting a longer mixing length. We have previously demonstrated that increasing the drive voltage on the piezoelectric transducers increased the amplitude of the acoustic waves, and increased the fluid mixing [6]. This was confirmed with Particle Image Velocimetry.

Piezoelectric transducers were driven at 15-40 Vpp. Bead mixing was achieved for 1, 3, 5, 6, and 10 μm polystyrene and glass beads at drive frequencies that correlated to piezoceramic resonance frequencies. Mixing was also achieved

with 1 μm diameter B.g. spores and 5 μm beads suspended in aqueous solution. As the level of mixing increased, we observed increased binding of B.g. to antibody coated beads [6].

Acoustic Concentration

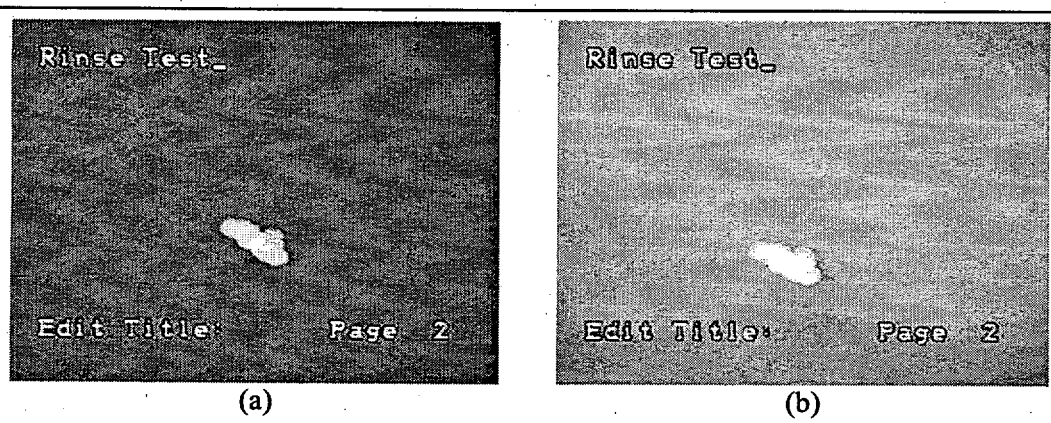
Particle concentration with two (or more) piezoelectric transducers required careful balance between transducers; an imbalance in radiation force resulted in mixing. We have since begun exploring alternate geometries to improve concentration efforts, including single element transducers in rectangular chambers and single-annular or arrayed-annular transducers in cylindrical chambers.

We have demonstrated acoustic concentration in flowing fluids using single element rectangular chambers. In this configuration, a standing wave was created by reflecting the acoustic wave off the opposing wall of the chamber. 5 μm diameter polystyrene particles were held in pressure nodes while water was pumped through the chamber at flow rates of up to 100 μl/min by a syringe pump. Faster flows caused particles to be dislodged from the pressure nodes. We continue to quantify the number of particles that can be trapped and held with this method. Early estimates are that approximately 50% of particles can be trapped, and once trapped remain stable in flow rates up to 100 μl/min. Having multiple trapping zones would improve trapping rates.

We are interested in using acoustic concentration to trap beads and enable bead washing. This is desirable, for example, when there is unbound fluorescent labeling dye in the background that decreases the signal to noise ratio. We have simulated an assay with a washing step by trapping beads in a solution of water and red dye. Once trapped, the chamber was flushed (50 μl/min) with plain water (no dye) to wash out the background signal. Figure 4 shows images of beads in solution pre- and post-washing, where post-wash has significantly decreased dye concentration. Flushing for a longer duration would virtually wash away all unbound dye.

3.2 Dielectrophoresis

We have used dielectrophoresis (DEP) to capture particles from a flowing stream. We have captured a variety of particles in homogenous solutions: Bacillus globigii spores, Erwinia herbicola bacteria, DNA, glass beads, and polystyrene beads. Most recently we have begun to collect particles in mixed solutions. We were able to remove a well-characterized background material, Arizona road dust, while allowing a purified solution of polystyrene beads to pass out of the chamber. Particle concentrations, as measured by the flow cytometer at the output of the DEP chamber, are shown in Figure 5. These plots show a dramatic decrease in dust at the output when the DEP electrodes are active because they dust collects on the electrodes. While maintaining excitation to the electrodes and upon flushing the chamber with water, some additional beads are recovered but virtually no dust. After turning off the DEP electrodes and again flushing with water, the dust releases from the electrodes and flows out of the chamber.



(a) (b)

Figure 4. Images of acoustically trapped beads a) pre-wash and b) post-wash. Washing greatly decreased concentration of dye in solution.

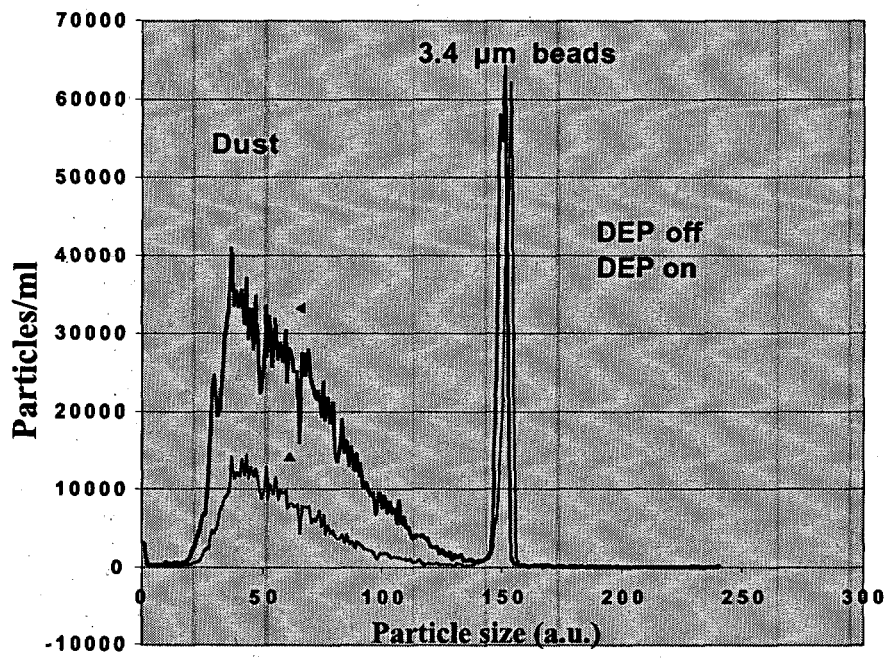


Figure 5. Quantification by flow cytometry of particle concentration at the output reservoir with or without Dielectrophoresis. DEP greatly reduced the amount of dust collected at the output while the amount of beads remained the same as with no DEP. Flushing with water while DEP electrodes are active does not dislodge the dust. Turning off the electrodes allows for release and recovery of dust.

4. CONCLUSION

We have developed an architecture and discrete components for automated sample preparation for biological assays. We are testing components and integrating them into a complete system. We have demonstrated the use of acoustic radiation pressure for sample and reagent mixing as well as for particle trapping and concentration. Acoustic mixing has improved the binding kinetics of beads and B.g. spores. Acoustic concentration has enabled washing of beads trapped in a flowing fluid. Dielectrophoresis, an alternative and complimentary technique, was shown to selectively trap particles in a flowing fluid. We demonstrated purification of a sample containing beads and dust. The dust was removed from the solution while the beads flowed through to the collection reservoir. In this fashion, DEP can be used to concentrate and purify select targets in mixed samples. The goal of these sample preparation techniques is to accept a dirty sample and output a concentrated and purified sample that requires less reagents, requires less time to perform assays, and improves sensitivity and specificity of assays. Examples of packaging and connecting devices have been demonstrated. Ultimately we intend to implement this fluidic handling system into pathogen detection systems for a variety of applications.

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